The International Journal of Biochemistry & Cell Biology 43 (2011) 358–362

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Golgi membranes from liver express an ATPase with femtomolar copper affinity, inhibited by cAMP-dependent protein kinase

Elaine Hilário-Souza, Rafael H.F. Valverde, Thiago Britto-Borges, Adalberto Vieyra, Jennifer Lowe*

Laboratório de Físico-Química Biológica Aída Hassón-Voloch, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal de Rio de Janeiro and Instituto Nacional de Biologia Estrutural e Bioimagem, 21941-902 Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 17 July 2010
Received in revised form 3 November 2010
Accepted 8 November 2010
Available online 21 November 2010

Keywords:
Copper ATPase
Copper affinity
Cell signaling
Regulatory phosphorylation
cAMP-dependent protein kinase

ABSTRACT

Copper-stimulated P-type ATPases are essential in the fine-tuning of intracellular copper. In the present work we characterized a copper-dependent ATPase hydrolysis in a native Golgi-enriched preparation from liver and investigated its modulation by cyclic AMP-dependent protein kinase (PKA). The very high-affinity Atp7b copper pump presented here shows a $K_{0.5}$ for free copper of 2.5×10^{-17} M in bathocuproine disulfonate/copper buffer and ATP hydrolysis was inhibited 50% upon stimulation of PKA pathway, using forskolin, cAMP or cholera toxin. Incubation with PKA inhibitor ($PKAi_{5-24}$ peptide) raises Cu(I)-ATPase activity by 50%. Addition of purified PKA α-catalytic subunit increases $K_{0.5}$ for free copper (6.2×10^{-17} M) without modification in the affinity for ATP in the low-affinity range of the substrate curve (~1 mM). The Hill coefficient for free copper activation also remains unchanged if exogenous PKA is added (2.7 and 2.3 in the absence and presence of PKA, respectively). The results demonstrate that this high-affinity copper pump in its natural environment is a target of the liver PKA pathway, being regulatory phosphorylation able to influence both turnover rate and ion affinity.

1. Introduction

Copper is an essential heavy-metal, which is central to the function of many enzymes involved in important metabolic pathways. An excess of copper is harmful because it enhances the production of free radicals. To avoid this deleterious event, copper homeostasis is carefully regulated (Kaplan and Lutsenko, 2009). In mammals, copper transport is carried out by two different P-type ATPases: (i) Atp7a, responsible for copper delivery to different tissues, and (ii) Atp7b, hepatic Cu(I)-ATPase responsible for the distribution of the metal to other tissues and export of excess copper into the bile (Lutsenko et al., 2008).

Several studies suggest a link between copper homeostasis and signaling events (Guo et al., 2005; Mercer et al., 2003; Michalczyk et al., 2008; Petris et al., 2002; Schlief et al., 2006; Veldhuis et al., 2009). Regulatory phosphorylation of the yeast Cu(I)-ATPase by cAMP-dependent protein kinase (PKA) has been demonstrated (Valverde et al., 2008). In mammals, kinase-mediated modulation of copper transport would be triggered by hormones and/or autacoids. Hormonal regulation of human Cu(I)-ATPases have been described (Michalczyk et al., 2008; Hardman et al., 2007), and has led to the hypothesis that receptor/kinase-mediated signaling is relevant in copper homeostasis.

Here we demonstrate that native Atp7b has a femtomolar copper affinity for catalytic phosphorylation and turnover, and that PKA – triggered by different hormones in liver (Walsh et al., 1968; Kuwahara et al., 1995) – is involved in modulation of Atp7b activity in its natural Golgi membrane environment.

2. Materials and methods

2.1. Reagents

Buffers, protease inhibitors, ATP, $PKAi_{5-24}$ and the polyclonal antibody against TGN-46 were supplied by Sigma Aldrich. ^{32}Pi was from IPEN. [γ-^{32}P]ATP was prepared as in Maia et al. (1983). The PKA α-catalytic subunit and the monoclonal antibody against GM130 were from Calbiochem. The antibodies against PKA α-catalytic subunit and Atp7b were purchased from Santa Cruz Biotechnology. Nitrocellulose membranes, ECL™ system and secondary antibodies were from GE Healthcare. Pig livers were obtained from a veterinarian-supervised slaughterhouse.

2.2. Preparation of Golgi-enriched membranes

This was performed as previously described (Subramaniam et al., 1992). Livers were transported in a chilled homogeniza-

Abbreviations: BCS, bathocuproine disulfonic acid; DTT, dithiothreitol; FSK, forskolin; CTX, cholera toxin; $PKAi_{(5-24)}$, PKA inhibitor 5–24-peptide.

* Corresponding author at: Avenida Carlos Chagas Filho 373-Bloco G, sala 37, Cidade Universitária, 21941-902 Rio de Janeiro, Brazil. Tel.: +55 21 2562 6520; fax: +55 21 2280 8193.
E-mail address: lowe@biof.ufrj.br (J. Lowe).

1357-2725 © 2010 Elsevier Ltd. Open access under the Elsevier OA license.
doi:10.1016/j.biocel.2010.11.004

tion buffer (HB) containing 250 mM sucrose, 20 mM Hepes–TRIS (pH 7.6), 5 mM $MgCl_2$, 1-phenylmethanesulfonyl fluoride, and 0.15 mg/ml of soybean trypsin inhibitor. Homogenization at 4 °C was carried out in 1.5 vol HB. After 10 min centrifugation at 10,000 × *g*, the pellet (unbroken cells, nuclei, mitochondria and membranes) was re-extracted with HB and centrifuged again. Supernatants from both spins were pooled and centrifuged at 100,000 × *g* for 1 h. The supernatant was decanted and the total membrane pellet was resuspended in 10 vol HB and recentrifuged at 100,000 × *g* for 40 min, this second spin enriched the Golgi markers. The membrane fractions were resuspended in MOPS–KOH (pH 7.6) containing 1 mM DTT and stored in liquid N_2.

2.3. Identification of Golgi markers and Atp7b by Western blotting

Protein concentration was estimated as described by Lowry et al. (1951). Between 50 and 100 μg of each sample was separated by SDS-PAGE (10%) and transferred to nitrocellulose membranes blocked with 5% milk, incubated overnight at 4 °C with the primary antibodies for Golgi markers (GM130, TGN46), PKA α-catalytic subunit or Atp7b, and subsequently incubated for 1 h with a secondary antibody-HRP conjugate at room temperature and developed with the ECL™ system.

2.4. Cu(I)-ATPase activity

The activity was assayed according to Fiske and Subbarow (1925). The assays contained 20 mM MOPS–KOH (pH 7.5), 100 mM K_2SO_4, 10 mM $MgSO_4$, 10 mM NaN_3, 10 mM NaF and 4% glycerol (w/v). Membranes (0.25 mg/ml) were incubated (30 min) on ice in (i) assay medium supplied with 100 μM ascorbate, 1 mM Na_2SO_3 and 50 μM DTT, or (ii) assay medium plus 300 μM BCS – the copper chelator previously used in Cu(I)-ATPase assays (Lowe et al., 2004) – without reducing agents. After equilibration at 37 °C, the reaction was started by adding 5 mM ATP or varying ATP concentrations, as specified. The ATP concentration dependence was investigated in the absence or presence of purified PKA α-catalytic subunit (2000 U/ml). Cu(I)-ATPase activity was calculated by the difference between Pi release in the presence or absence of 300 μM BCS. Note that only contaminant copper (70–80 nM) was present. Pi release increased linearly during the first 20 min; therefore, measurements were stopped after 5 min with trichloroacetic acid (5% w/v) and ammonium molybdate. To exclude a possible contribution of other ion pumps to the BCS-sensitive activity, Na^+,K^+-ATPase and Ca^{2+}-ATPase were measured in the presence or absence of BCS. The ouabain- and EGTA-sensitive activities were barely detectable in the Cu(I)-ATPase assay conditions and no effect of BCS was observed on basal ATP hydrolysis.

2.5. Dependence of Cu(I)-ATPase activity on free copper concentration

Since contaminant copper in our solutions is enough for maximal Cu(I)-ATPase activity, the free copper concentration dependence was assayed in the presence of a BCS–copper buffer after removal of any contaminant copper. Membranes (0.25 mg/ml) were incubated on ice with BCS in 20 mM MOPS (pH 7.5), 100 mM K_2SO_4, 10 mM $MgSO_4$, 10 mM NaN_3, 10 mM NaF and 4% glycerol (w/v). After 30 min, the membrane suspensions were spun down at 18,000 × *g* (15 min; 4 °C) and the wet pellets suspended in the assay buffer for ATPase (0.2 ml, 37 °C) with different concentrations of $CuCl_2$ (plus 100 μM ascorbate, 1 mM Na_2SO_3 and 50 μM DTT) to give the desired free copper concentrations, or in buffer supplied with BCS alone without reducing agents. The free copper concentration dependence was also studied in the absence or presence of exogenous soluble PKA α-catalytic subunit (2000 U/ml). The reactions were stopped 5 min after the addition of 5 mM ATP. Free copper was calculated by measuring the remaining BCS in the water volume of the pellet (and therefore in the final assay), assuming an association constant of 10^{20} for the $Cu(BCS)_2^{3-}$ complex (Miras et al., 2008). Given the water volume in the pellet (7.6 ± 0.6 μl) and the initial BCS concentration (300 μM), the final BCS in the assay (0.2 ml) was 11.4 μM. Cu(I)-ATPase activity was calculated by the difference between Pi release in the presence of BCS–copper buffer and the presence of 300 μM BCS alone.

2.6. Determination of wet volume in the pellet of BCS-treated membranes

After the treatment of membrane fragments with BCS, centrifugation and careful removal of the supernatant, the wet pellet was desiccated at 105 °C until constant dry weight (24 h). The volume of distribution of remaining BCS in pellets (7.6 ± 0.6 μl, $n = 4$) was calculated by the difference between the wet and dry weights.

2.7. Influence of PKA pathway modulators on Cu(I)-ATPase activity

Cu(I)-ATPase activity was measured in the presence of different activators (forskolin, cAMP, PKA α-catalytic subunit and cholera toxin) or the specific inhibitor ($PKAi_{5-24}$) of the PKA pathway.

2.8. Phosphorylation by [γ-^{32}P]ATP

Catalytic phosphorylation was performed as in Lowe et al. (2004) in the absence or presence of 250 μM BCS. Only contaminant copper was present. [γ-^{32}P]ATP was added at 5 μM (~1500 cpm/pmol).

2.9. Statistical analysis of the data

Each class of experiments was performed in triplicate with at least 4 independent preparations. The data were analyzed by one-way variance analysis. The significance of the differences was verified by the Bonferroni *t*-test ($p < 0.05$).

3. Results and discussion

3.1. Identification of Cu(I)-ATPase in membrane fractions of porcine liver

Golgi enrichment in the membrane fractions was confirmed by immunoblotting of GM130 (Fig. 1A) and TGN 46 (Fig. 1B), the markers for the *cis*- and *trans*-Golgi regions, respectively (Ponnambalam et al., 1996; Prescott et al., 1997). The markers did not appear in the initial homogenate, being detected after the last centrifugation and especially in the final sediment. Anti-Atp7b, a specific antibody, was also used to identify mammalian Cu(I)-ATPase (Fig. 1C).

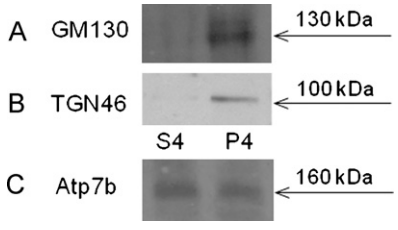

Fig. 1. Identification of Golgi markers and Atp7b. Representative immunoblottings of membranes probed with antibodies against GM130 (1:2000) (A), TGN46 (1:4000) (B) and Atp7b (1:1000) (C). S4 and P4 (A, B) correspond, respectively, to the last supernatant and pellet. (C) 2 different preparations.

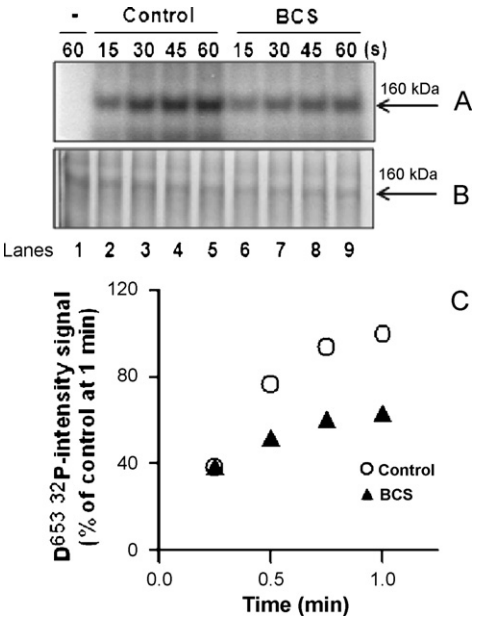

Fig. 2. Time-course of Atp7b catalytic phosphorylation. (A) Representative autoradiogram. Lane 1: sample denaturated before 60 s phosphorylation. Lanes 2–5: different phosphorylations times. Lanes 6–9: samples incubated with 250 μM BCS (30 min) and then phosphorylated. (B) Coomassie-blue staining of the same gel. (C) Densitometric representation of the phospho-signal/coomassie-blue staining ratio.

3.2. Catalytic phosphorylation of Cu(I)-ATPase

Formation of the acyl-phosphate intermediate of Atp7b and its sensitivity to copper ions are shown in Fig. 2. Catalytic phosphorylation was inhibited by 40% in the presence of BCS when a steady level was reached (1 min). Since Atp7b is the only P-ATPase with 160 kDa, the BCS-resistant phosphoenzyme probably represents a parcel of Atp7b activity linked with the occupancy of an occluded copper binding site in the membrane moiety. With the use of heterologously expressed Atp7b (Lutsenko et al., 2002), it has been proposed that copper sites inaccessible to BCS can sustain basal phosphorylation and enzyme turnover.

3.3. PKA pathway modulates Cu(I)-ATPase activity in native Atp7b

In previous work, phosphorylation by PKA of yeast Cu(I)-ATPase was shown to trigger a signal that determined the exchange between the phosphoryl group of EP and copper in chemical potential during catalysis (Valverde et al., 2008). In the present work we investigated whether an effect of PKA exists on Atp7b.

Golgi-enriched preparations contain a membrane associated PKA (Fig. 3, inset) that can modulate Atp7b in its natural environment, shown by the effect of specific activators and inhibitors (Fig. 3, main panel). Forskolin, which permanently activates the Golgi-localized adenylyl cyclase (Cheng and Farquhar, 1976), cAMP that activates the endogenous PKA, and purified exogenous α-catalytic subunit which phosphorylates in a cAMP-independent manner, can each inhibit Cu(I)-ATPase activity by 50%. Since modulation is mimicked by cholera toxin, it is clear that inhibition is a G_s protein-mediated event. Inversely, the highly specific inhibitor peptide of PKA ($PKAi_{5-24}$), increases Cu(I)-ATPase activity by 50%. This mirror image of the inhibition obtained with activators indicates that endogenous cAMP produced by endogenous adenylyl cyclase is sufficient to partially inhibit the control activity by activating the membrane-associated PKA immunodetected in the experiment depicted in the inset of Fig. 3. Therefore, stimulation

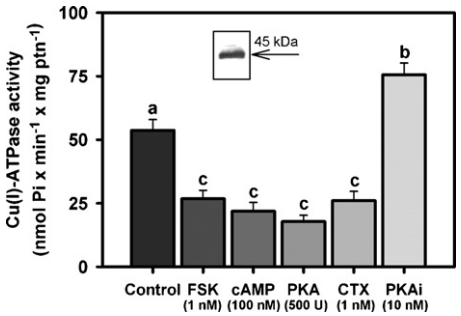

Fig. 3. Modulation of Cu(I)-ATPase activity by PKA. Cu(I)-ATPase activity was measured in the presence of forskolin (FSK), cAMP, PKA α-catalytic subunit (0.25 ml assay), cholera toxin (CTX), or $PKAi_{5-24}$. Different lowercase letters above the bars indicate statistically significant differences. Inset: representative immunoblotting of PKA α-catalytic subunit in the membranes probed against anti-PKA antibody (1:1000).

of Atp7b by $PKAi_{5-24}$ is likely due to inhibition of the endogenous PKA.

3.4. Dependence of Cu(I)-ATPase activity on ATP and free copper concentrations

Copper concentration dependence of Cu(I)-ATPase activity in assays where a BCS–copper buffer were present after treatment of the membranes with BCS to remove the original contaminant metal is shown in Fig. 4A. Using BCS–copper buffer, we measured a $K_{0.5}$ for free copper of 2.5×10^{-17} M. In contrast with that found in bacteria (Mandal et al., 2002; Hatori et al., 2008), this high affinity probably reflects a functional adaptation for efficient copper trafficking among copper-binding proteins in concentrations well below those toxic to mammalian cells or, in other words, with virtually no free copper (Banci et al., 2010). Fig. 4A also shows a sigmoidal dependence on copper concentration (Hill coefficient = 2.7 ± 0.4), indicating a positive cooperativity in copper binding and activation. Positive cooperativity was found in phosphorylation and copper transport with heterologously expressed Atp7a (Hung et al., 2007), supporting the view that interaction between copper-binding sites might be a general feature of Cu(I)-ATPases.

In the presence of purified soluble PKA, enough for maximal inhibitory effect (2000 U/ml), Atp7b shows a reduced affinity for copper. The $K_{0.5}$ value for free copper increases 2.5 fold (to 6.2×10^{-17} M; $p < 0.05$), thus demonstrating that regulatory phosphorylation induces a conformational change at copper binding sites. This is likely a result of long-range intramolecular communications between ion transport domains and regulatory PKA sites. In contrast, there is no modification ($p = 0.67$) in the Hill coefficient of the free copper curve with exogenous PKA (2.3 ± 0.7), suggesting that regulatory phosphorylation leads to a decreased affinity without modification in the stoichiometry of copper transport per cycle.

Due to the very low $K_{0.5}$ for free copper, and taking into account that copper contamination in the solutions ranges 70–80 nM, the following experiments were carried out without the addition of copper salts. The ATP concentration dependence of Cu(I)-ATPase activity shows Michaelian behavior (Fig. 4B). The V_{max} (69.1 ± 4.3 nmol Pi × mg^{-1} × min^{-1}), which is remarkably similar to that found with BCS-treated enzyme then supplied with saturating free copper (Fig. 4A), indicates a higher steady-state ATPase activity compared with other studies using both native and heterologously expressed mammalian Cu(I)-ATPases (Pilankatta et al., 2009; Takeda et al., 1999). The K_m for ATP of native Atp7b in its natural environment is 0.97 ± 0.22 mM, which is similar to that found with detergent-solubilized CopA (Mandal et al., 2002). In contrast with that found for copper, the affinity for ATP remained unchanged

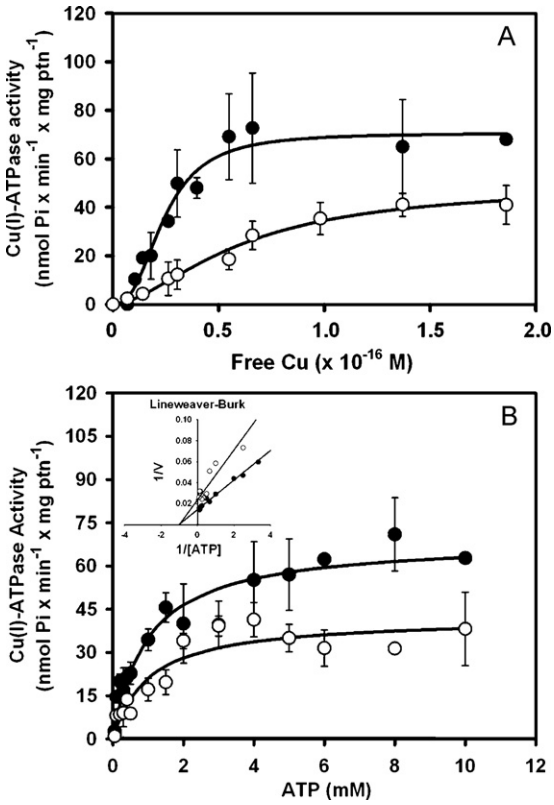

Fig. 4. Free copper and ATP dependence. (A) Cu(I)-ATPase activity was measured in different free copper concentrations. The closed symbols represent control conditions (no exogenous PKA) and open symbols correspond to experiments in the presence of exogenous PKA α-catalytic subunit. (B) ATP concentration dependence of Cu(I)-ATPase activity in the absence (closed symbols) or presence (open symbols) of PKA α-catalytic subunit. The equations $v = V_{max} \times [\text{free Cu}]^n/(K^n_{0.5} + [\text{free Cu}]^n)$ (A) and $v = V_{max} \times [\text{ATP}]/(K_m + [\text{ATP}])$ (B) were used to calculate the kinetic parameters. Inset: Lineweaver–Burk plot visualizing the K_m and V_{max} for ATP. The kinetic parameters are mean ± SEM of the values obtained by fitting the functions to the experimental points of each determination carried out with different membrane preparations.

when PKA α-catalytic subunit is present ($K_m = 1.00 \pm 0.31$ mM; $p = 0.69$) whereas V_{max} decreased to 42.1 ± 3.6 nmol Pi mg^{-1} min^{-1} ($p < 0.05$).

Since steady levels of phosphorylated intermediate in Atp7b are formed with the use of 1–50 μM ATP in different assays (Pilankatta et al., 2009; Tsivkovskii et al., 2002; see Fig. 2), the submillimolar affinity of Cu(I)-ATPase in Atp7b may be associated with nucleotide action in the overall turnover of the pump (Kühlbrandt, 2004). Since PKA-mediated phosphorylation affects the turnover rate of the ATPase without modification in the affinity for ATP, it may be that the intramolecular signal triggered from PKA inhibitory sites (or site) decreases the rate of breakdown of the phosphorylated intermediate during the catalytic cycle without change in ATP binding.

In conclusion, these results support the hypothesis that there is a direct PKA-mediated modulation of Atp7b in Golgi liver membranes, and that inhibition of its turnover is a consequence of triggering the PKA signaling pathway. The messengers that activate PKA would be hormones and autacoids capable of modulating active copper transport through rapid kinase-mediated processes. Expression of human Cu(I)-ATPases and copper transport were modulated by hormones, such as insulin, oestrogen and prolactin, in different cell types (Hardman et al., 2007; Kelleher and Lönnerdal, 2006) that lead to modifications mediated by different protein kinases, as demonstrated here for PKA. Since non-specific protein phosphatases strongly decreases Atp7b activity (data not shown) it is clear that, besides the inhibitory action of PKA, stimulatory kinases are also involved in a complex network that regulates the liver Cu(I)-ATPase. The possibility of hormones acting on protein kinases in liver to modulate active copper transport opens new horizons in our understanding of how cells and organisms handle intracellular copper.

Acknowledgements

This work was supported by grants from CNPq, CAPES and FAPERJ (Brazil). We express our appreciation for the support given by our laboratory colleagues, and Andrea Abrantes for her technical support. We especially acknowledge Dr. Elisabeth Mintz (CEA, Grenoble, France) for calculating free copper and BioMedES for corrections in the presentation of the manuscript.

References

Banci L, Bertini I, Ciofi-Baffoni S, Kozyreva T, Zovo K, Palumaa P. Affinity gradients drive copper to cellular destinations. Nature 2010;465:645–8.
Cheng H, Farquhar MG. Presence of adenylate cyclase activity in Golgi and other fractions from rat liver. II. Cytochemical localization within Golgi and ER membranes. J Cell Biol 1976;70:671–84.
Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. J Biol Chem 1925;66:375–400.
Guo Y, Nyasae L, Braiterman LT, Hubbard AL. NH_2-terminal signals in ATP7B Cu-ATPase mediate its Cu-dependent anterograde traffic in polarized hepatic cells. Am J Physiol Gastrointest Liver Physiol 2005;289:G904–16.
Hardman B, Michalczyk A, Greenough M, Camakaris J, Mercer JF, Ackland ML. Hormonal regulation of the Menkes and Wilson copper-transporting ATPases in human placental Jeg-3 cells. Biochem J 2007;402:241–50.
Hatori Y, Hirata A, Toyoshima C, Lewis D, Pilankatta R, Inesi G. Intermediate phosphorylation reactions in the mechanism of ATP utilization by the copper ATPase (CopA) of *Thermotoga maritima*. J Biol Chem 2008;283:22541–9.
Hung YH, Layton MJ, Voskoboinik I, Mercer JF, Camakaris J. Purification and membrane reconstitution of catalytically active Menkes copper-transporting P-type ATPase (MNK; ATP7A). Biochem J 2007;401:569–79.
Kaplan JH, Lutsenko S. Copper transport in mammalian cells: special care for a metal with special needs. J Biol Chem 2009;284:25461–5.
Kelleher SL, Lönnerdal B. Mammary gland copper transport is stimulated by prolactin through alterations in Ctr1 and Atp7A localization. Am J Physiol Regul Integr Comp Physiol 2006;291:R1181–91.
Kühlbrandt W. Biology, structure and mechanism of P-type ATPases. Nat Rev Mol Cell Biol 2004;5:282–95.
Kuwahara M, Fushimi K, Terada Y, Bai L, Marumo F, Sasaki S. cAMP-dependent phosphorylation stimulates water permeability of aquaporin-collecting duct water channel protein expressed in *Xenopus* oocytes. J Biol Chem 1995;270:10384–7.
Lowe J, Vieyra A, Catty P, Guillain F, Mintz E, Cuillel M. A mutational study in the transmembrane domain of Ccc2p, the yeast Cu(I)-ATPase, shows different roles for each Cys-Pro-Cys cysteine. J Biol Chem 2004;279:25986–94.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.
Lutsenko S, Efremov RG, Tsivkovskii R, Walker JM. Human copper-transporting ATPase ATP7B (the Wilson's disease protein): biochemical properties and regulation. J Bioenerg Biomembr 2002;34:351–62.
Lutsenko S, Gupta A, Burkhead JL, Zuzel V. Cellular multitasking: the dual role of human Cu-ATPases in cofactor delivery and intracellular copper balance. Arch Biochem Biophys 2008;476:22–32.
Maia JCC, Gomes SL, Juliani MH. Preparation of (gamma-^{32}P) and (alpha-^{32}P)-nucleoside triphosphates with high specific activity. In: Morel CM, editor. Genes of parasites: a laboratory manual. Rio de Janeiro, Brazil: Editora Fundação Oswaldo Cruz; 1983. p. 146–67.
Mandal AK, Cheung WD, Argüello JM. Characterization of a thermophilic P-type Ag^+/Cu^+-ATPase from the extremophile *Archaeoglobus fulgidus*. J Biol Chem 2002;277:7201–8.
Mercer JF, Barnes N, Stevenson J, Strausak D, Llanos RM. Copper-induced trafficking of the Cu-ATPases: a key mechanism for copper homeostasis. Biometals 2003;16:175–84.
Michalczyk A, Bastow E, Greenough M, Camakaris J, Freestone D, Taylor P, et al. ATP7B expression in human breast epithelial cells is mediated by lactational hormones. J Histochem Cytochem 2008;56:389–99.
Miras R, Morin I, Jacquin O, Cuillel M, Guillain F, Mintz E. Interplay between glutathione, Atx1 and copper. 1. Copper(I) glutathionate induced dimerization of Atx1. J Biol Inorg Chem 2008;13:195–205.
Petris MJ, Voskoboinik I, Cater M, Smith K, Kim BE, Llanos RM, et al. Copper-regulated trafficking of the Menkes disease copper ATPase is associated with formation of a phosphorylated catalytic intermediate. J Biol Chem 2002;277:46736–42.
Pilankatta R, Lewis D, Adams CM, Inesi G. High yield heterologous expression of wild-type and mutant Cu^+-ATPase (ATP7B, Wilson disease protein) for functional

characterization of catalytic activity and serine residues undergoing copper-dependent phosphorylation. J Biol Chem 2009;284:21307–16.
Ponnambalam S, Girotti M, Yaspo ML, Owen CE, Perry AC, Suganuma T, et al. Primate homologues of rat TGN38: primary structure, expression and functional implications. J Cell Sci 1996;109:675–85.
Prescott AR, Lucocq JM, James J, Lister JM, Ponnambalam S. Distinct compartmentalization of TGN46 and beta 1,4-galactosyltransferase in HeLa cells. Eur J Cell Biol 1997;72:238–46.
Schlief ML, West T, Craig AM, Holtzman DM, Gitlin JD. Role of the Menkes copper-transporting ATPase in NMDA receptor-mediated neuronal toxicity. Proc Natl Acad Sci USA 2006;103:14919–24.
Subramaniam VN, bin Mohd Yusoff AR, Wong SH, Lim GB, Chew M, Hong W. Biochemical fractionation and characterization of proteins from Golgi-enriched membranes. J Biol Chem 1992;267:12016–21.
Takeda K, Ushimaru M, Fukushima Y, Kawamura M. Characterization of a P-type copper-stimulated ATPase from mouse liver. J Membr Biol 1999;170: 13–6.
Tsivkovskii R, Eisses JF, Kaplan JH, Lutsenko S. Functional properties of the copper-transporting ATPase ATP7B (the Wilson's disease protein) expressed in insect cells. J Biol Chem 2002;277:976–83.
Valverde RH, Morin I, Lowe J, Mintz E, Cuillel M, Vieyra A. Cyclic AMP-dependent protein kinase controls energy interconversion during the catalytic cycle of the yeast copper-ATPase. FEBS Lett 2008;582:891–5.
Veldhuis NA, Valova VA, Gaeth AP, Palstra N, Hannan KM, Michell BJ, et al. Phosphorylation regulates copper-responsive trafficking of the Menkes copper transporting P-type ATPase. Int J Biochem Cell Biol 2009;41:2403–12.
Walsh DA, Perkins JP, Krebs EG. An adenosine 3′,5′-monophosphate-dependant protein kinase from rabbit skeletal muscle. J Biol Chem 1968;243:3763–5.